Comm.
Dr. Jacobson
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CHRONIC PULMONARY DISEASES

#914

THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.
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Application for Research Grant
(Use extra pages as needed)

Date: May 17, 1973

1. Principal Investigator (give title and degrees):

Michael E. Lamm, M.D.
Associate Professor of Pathology

2. Institution & address:

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3. Department(s) where research will be done or collaboration provided:

Pathology

4. Short title of study:

Immune Mechanisms of Mucous Membranes

5. Proposed starting date: January 1, 1974

6. Estimated time to complete: 3 years

7. Brief description of specific research aims:

a. Secretory immunoglobulin A (IgA*), the principal class of antibody in mucous membrane secretions, serves as a first-line defense system at the interface between the interior of the body and the outside world. The unique portion of the secretory IgA molecule is the secretory component (SC) which is not present in any other immunoglobulin, including serum-type IgA. We want to characterize further the SC portion of human exocrine IgA by doing amino acid sequence determinations of selected regions, initially the N-terminus and the regions containing the cysteine residues which form the interchain disulfide bridges. We want to determine the number of disulfide bridges which join SC to the remainder of the molecule and where they are located. For example, is the SC joined to the alpha chain and, if so, what portion?

b. The reason that IgA is the most abundant class of antibody in mucous membrane secretions (whereas in serum it is only a minor component) is the large number of IgA-type plasma cells in mucous membranes compared to cells synthesizing other classes of antibody. We want to gain insights into the mechanisms responsible for the preponderance of IgA-producing immunocytes in mucous membranes.

* Abbreviations: IgA, immunoglobulin A; SC, secretory component; BSC and FSC, bound and free secretory component.

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c. Secretory IgA presents a unique instance among proteins of a molecule of which some components (H,L, and J chains) are synthesized by one type of cell (plasma cell) and another component (SC) by a different type of cell (epithelial cell). We want to investigate the locus where SC is coupled to the remainder of the molecule to give fully assembled secretory IgA, e.g., within, between, or on the surface of the epithelial lining cells of mucous membranes.

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8. Brief statement of working hypothesis:

Secretory IgA plays a key role in host defense, but its chemical structure is much less well understood than that of IgG or IgM, the other principal immunoglobulins. We want to focus on its SC, the portion which renders secretory IgA unique compared to general immunoglobulin structure, in terms of both structure and function. We plan to investigate the hypothesis that SC, which is elaborated by the epithelial lining cells of mucous membranes, serves as a local receptor to capture circulating bone marrow derived (B) lymphocytes that happen to have IgA on their surface. By this means, the preponderance of IgA-type plasma cells in mucous membranes, which is the cellular basis of the secretory IgA system, could be explained.

9. Details of experimental design and procedures (append extra pages as necessary)

A great deal of evidence indicates that local immune mechanisms in mucous membrane secretions are extremely important for defense against a variety of common infectious diseases and furthermore that the antibodies in mucous membrane secretions can be considered as a system which is distinct from circulating antibody (1,2). Thus, in quantitative terms the main immunoglobulin class in secretions is IgA, which is but a minor component of the humoral immunoglobulins where IgG predominates, and in qualitative terms there are differences in the structure of secretory vs. serum IgA. The former occurs as an 11S protein containing 2 four-chain subunits ($[\alpha_2L_2]_2$) plus an SC chain and a J chain (3) whereas the latter occurs mostly as a 7S molecule (α_2L_2).

We are interested in the SC portion of the secretory IgA molecule because it is distinctive, occurring normally only in secretory IgA, or in a free form in secretions, and being absent from serum. When mixed in vitro with a mixture of immunoglobulins of different classes, it associates preferentially with dimeric IgA (4). (The J chain is not unique to IgA since it occurs in all polymeric immunoglobulins, e.g., polymeric serum-type IgA and IgM). A number of functions have been suggested for the SC portion of secretory IgA such as a role in transporting secretory IgA across epithelial surfaces and protection against proteolysis, but in fact there is presently no compelling evidence for any of the proposals, although there is support for the latter.

Until recently information on the chemical structure of SC has lagged for lack of satisfactory isolation procedures. However, we (5) and Kobayashi (6) have devised methods for purifying human BSC and FSC, and O'Daly and Cebra (7) have been successful with the rabbit counterparts. Our method for isolating BSC is entirely biochemical, the key step being a cyanogen bromide cleavage of the whole secretory IgA molecule, which does not affect the SC since it lacks methionine, whereas Kobayashi's procedure employs an immunoadsorbent. The purification of rabbit BSC has different requirements since unlike the human protein it is non-covalently linked to the remainder of the molecule. Both we and Kobayashi have found human BSC and FSC to be similar in terms of molecular weight, antigenic determinants, and amino acid composition. In addition, we have shown (unpublished) that their tryptic peptide maps and N-terminal amino acid sequences are similar. Thus, it is likely that BSC and FSC are two different forms of the same protein. Whether the FSC has an independent function aside from its role as part of secretory IgA is unknown.

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In addition to the unique features of the secretory IgA molecule, the secretory antibody system presents a number of interesting facets in terms of cellular immunology. For example, secretory IgA is to my knowledge a unique instance of a multi-chained protein of which different portions are synthesized in different cell types, the alpha, L and J chains being secreted by the immunocytes in the mucous membranes whereas the SC is made in the overlying epithelial cells (1,8). The site where SC is complexed to form the complete secretory IgA molecule is not known with certainty, whether between epithelial cells, within epithelial cells, or on the surface of the mucous membrane. Secondly, the reason for the large amount of secretory IgA in mucous membrane secretions can be traced to the abundance of immunocytes in mucous membranes which contain IgA (9-11). In the spleen and peripheral lymph nodes, in contrast, IgA-containing immunocytes are in the minority and most immunocytes contain IgG. The mechanisms which account for this preferential location of IgA-producing immunocytes in mucous membranes are not understood. Two general possibilities come to mind: (1) IgA producers or cells destined to produce IgA preferentially home to mucous membranes or (2) a local inductive influence in the mucous membranes either causes immunocytes preferentially to differentiate into IgA-producers or causes IgA-immunocytes to proliferate more rapidly as compared to other anatomical locations.

There is good reason to believe that the secretory IgA system is of fundamental importance for protecting the body against the entry of potentially pathogenic microorganisms. One of the requisites for understanding this system better is a more complete knowledge of the chemical structure and function of the secretory IgA molecule. To my mind the most interesting portion of this molecule is the SC, which renders it unique when compared with the structure of other immunoglobulins. We have published (5) the only satisfactory procedure for purifying human BSC on a preparative scale, and to my knowledge no other laboratory is actively engaged in the same kind of detailed studies of its chemical structure. (In contrast, the J chain, which we are not studying, is being investigated in a number of laboratories). I think that isolation of BSC via immunoadsorbents, as used by Kobayashi (6) is not so satisfactory for preparative work. This reasoning is consistent with the fact that most of the analytical studies in Kobayashi's paper were done on FSC, and not on BSC which was probably obtained in much more limited quantities. Now that we have developed procedures for isolating BSC, we would like to characterize it more fully. In addition, we want to investigate the mechanisms responsible for the predominance of IgA-immunocytes in mucous membranes and to inquire whether SC plays a role.

Methods of Procedure

1. Amino acid sequence studies

Knowledge of the amino acid sequence of SC is of current interest for two reasons. First, we would like to know if BSC and FSC, which are similar in terms of criteria already studied in our laboratory (size, antigenic determinants, composition and peptide maps) are identical, and the ultimate criterion is, of course, the primary structure. Second, we want to know whether there is any homology to the sequence of immunoglobulin H and L chains. Presumably the evolutionary development of SC is unrelated to that of H and L chains since the pertinent cell types (immunocytes vs. epithelial cells) are so different. However, again the ultimate criterion is the amino acid sequence. Comparison of the sequence of SC with the sequences of other proteins may shed light on its evolutionary origin.

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SC, as we (5) and Kobayashi (6) have shown, has a molecular weight of 71,000 daltons. The largest proteins (immunoglobulin or otherwise) sequenced thus far are a human gamma chain, 53,000 daltons (by Edelman's group) and a mu chain, 70,000 daltons (by Putnam's group). In both cases the proteins, being from patients with multiple myeloma, were available in unlimited amounts and were easily purified by standard techniques. Furthermore, the investigators could be guided by homologies to previously sequenced L chains and portions of H chains. Even so the sequence determinations required several years. In contrast, in our own work on SC we have no myeloma protein sources and only modest supplies of colostrum. We have first to isolate secretory IgA and then from it isolate SC. Also, since human SC is devoid of methionine, it would not be possible to work with cyanogen bromide fragments of the parent protein, a procedure which usually simplifies sequence studies. In conclusion, because of its large size and lack of ready availability in large quantities, I do not believe it will be feasible in the near future to attempt a total sequence of SC.

On the other hand I do believe that SC is a significant and interesting protein and that important information can still be obtained by amino acid sequence studies of selected portions, which we are doing. To my knowledge no other laboratory is engaged in such studies on human SC.

a. N-terminus

We have found that both BSC and FSC are amenable to automated sequence analysis from the N-terminus. (In this regard human SC may be different from the rabbit counterpart in which an N-terminal residue could not be identified (7)). In collaboration with Dr. E.C. Franklin's laboratory (where the automated sequencer is located) we have determined the first 21 residues of human BSC and FSC, which are identical:

Lys-Ser-Pro-Ile-Phe-Gly-Pro-Glu-Glu-Val-Asp-Ser-Val-Glu-Gly-Gly-Ser-Thr-( )-Thr-Ser-

This is the only sequence information available on human SC. There are no obvious homologies to H and L chains, which is in keeping with the synthesis of SC by a different cell type, the epithelial cell. We are attempting to extend this N-terminal sequence further into the molecule.

b. Disulfide links to the remainder of secretory IgA molecule

We and others have shown that, unlike rabbit SC, human SC is covalently linked to the remainder of the molecule and can be liberated only after partial or complete reduction. To which chain or chains the SC is joined (e.g., alpha, J or L) and the number of such links are unknown. We want to investigate these questions.

1) Number of interchain disulfide bridges

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SC is isolated after partial reduction and alkylation with radioactive iodoacetic acid. If it is assumed that only interchain disulfide bridges are cleaved under mild conditions, the number of counts incorporated into SC and/or the number of S-carboxymethylcysteine residues per mole after amino acid analysis will reveal the number of interchain disulfide bonds between SC and the remainder of the molecule. It is, of course, possible for labile intrachain bridges to be reduced under mild conditions, which would lead to an overestimation, but in general the assumption holds. Certainly all the interchain bridges will be split because mild

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reduction is sufficient to liberate SC. Our initial results show that 2 disulfide bridges form the interchain covalent links to SC.

2) Sequences around the interchain disulfide bridges of SC

SC is isolated after mild reduction and radioactive alkylation with ^{14}C-iodoacetic acid, which marks the interchain cysteines. In order to denature SC preparatory to enzyme digestion, interchain bridges are broken by complete reduction and the sulfhydryl groups of the interchain cysteines are blocked with cold iodoacetamide. The completely reduced and alkylated SC is then digested with enzymes to produce short polypeptides. Initially we shall be studying peptic-tryptic digests (a combination that has proved suitable in the past). The radioactive peptides containing the interchain cysteine residues are isolated by appropriate methods (gel filtration, ion exchange or paper chromatography, or high voltage electrophoresis on paper). The last method works well for small charged peptides, and the extra negative charge conferred on the interchain cysteines by the alkylation with iodoacetic acid proves advantageous. The various steps during the isolation of the radioactive peptides are followed by scintillation counting, and in the case of high-voltage electrophoresis on paper by autoradiography on x-ray film. Purified peptides are sequenced by the dansyl-Edman and/or subtractive Edman techniques.

3) Pairing of the interchain disulfide bridges

The approach discussed above in part 2) is the most sensitive and efficient way to isolate those cysteine peptides of SC which partake in interchain bridges. However, it gives no information on the pairing of these peptides (i.e., to which particular cysteine in the remainder of the secretory IgA molecule is each interchain cysteine of SC joined) because the interchain disulfide bridges are cleaved in the initial mild reduction required to introduce the radiolabel. One approach to this question is to do a cyanogen bromide digestion of the whole secretory IgA molecule (which will not affect SC since it lacks methionine) without prior reduction, and then isolate SC-containing fractions using our specific antisera as guides. The object then is to determine which other portion of the parent molecule is still attached to SC by disulfide bridges. This approach will be simple or complex depending on the location of methionine residues (e.g., in alpha chain) with respect to the disulfide bridge forming the link (e.g., from alpha chain) to SC. Preliminary work indicates that the first methionine from the N-terminus of the alpha chain occurs C-terminal to where SC and L chains join so that SC is part of a large fragment containing much of the alpha chains and L chains.

Two additional approaches can be used to determine the arrangement of the bridges. One is diagonal electrophoresis (12) in which a proteolytic digest of a protein is electrophoresed on paper, after which the disulfide bridges are cleaved directly on the paper by performic acid vapor (oxidation of cystine to cysteic acid). Then a second electrophoresis, under identical conditions to the first, is done in a direction perpendicular to the first dimension. Cysteic acid peptides appear off the "diagonal", their mobility, unlike the other peptides, having been altered by the oxidation, and can be isolated. The two halves of the same disulfide bridge will be located in the same band, and thus the nature of the cysteine pairings can be determined. In this method all the cysteine peptides in the secretory IgA molecule will be affected, and we are mainly concerned with those joining SC to the remainder of the molecule. It should be possible, however, to focus on the appropriate peptides in the diagonal maps because from the studies described in part 2) we would know the electrophoretic mobilities and sequences of the interchain cysteine peptides of SC. If these last can be identified in the diagonal maps, their partners would contain the cysteines to which they had been joined in the native secretory IgA molecule. These latter cysteic acid peptides would then be purified and sequenced.

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In addition to diagonal electrophoresis proteolytic digests of whole secretory IgA could be fractionated by conventional techniques and those peptides containing cystine bridges purified. Among these cystine peptides would be the cysteine residues identified in part 2). Their partners would contain the cysteine residue elsewhere in the secretory IgA molecule to which they are joined in the native protein.

In our laboratory we thus hope to determine which cysteine residues in SSC participate in interchain bridges and to which cysteines elsewhere in the molecule they are joined. For each partner of an interchain SC cysteine residue we might not know (depending on how the above work turns out) from which portion of which chain (?alpha or J) it originated. These questions could be answered by isolating radioactive cysteine peptides from the other component chains of secretory IgA after partial reduction and radioactive alkylation. However, we are not primarily interested in the alpha and J chains, and other investigators, including Frangione and Franklin at this institution, are actively studying these portions of the IgA molecule. Hopefully our data could be meshed with data on cysteine peptides from alpha and J chains obtained by others to answer questions of mutual interest.

2. Histological studies

As mentioned earlier, there is general agreement (e.g., 11,13) mostly from work with fluorescent antisera, that alpha and L chains (i.e., serum-type IgA) are secreted by plasma cells in the lamina propria of mucous membranes and that SC is made by the overlying epithelial cells. However, different workers disagree on the extent of alpha chain staining within epithelial cells. Thus, the actual route of transport to the lumen is still not understood (i.e., between epithelial cells or through epithelial cells) nor is the locus of coupling of SC to the serum-type IgA to yield secretory IgA known (i.e., between or within epithelial cells, or in the lumen).

Poger and I are studying this question in a novel way. In Ouchterlony plates with antisera made against secretory IgA and FSC, we observed reactions of partial identity which showed that SC in secretory IgA and FSC have distinct as well as shared antigens, presumably conformationally dependent. With appropriate insoluble immunoadsorbents we have been able to render anti-FSC entirely specific for FSC (i.e., no reaction with secretory IgA) and anti-BSC entirely specific for the bound conformation (no reaction with FSC). In the former instance anti-FSC is absorbed with secretory IgA, and in the latter instance anti-secretory IgA is absorbed with serum-type IgA, normal serum, and FSC. These absorbed antisera have been fluoresceinated so that we possess reagents specific for SC in its bound vs. free state, and we have been examining human tissue obtained from surgical specimens. We find that FSC occurs in epithelial cells from the Golgi zone (where the most brilliant fluorescence occurs) to the apex, but not at the base. BSC appears to be present only in the apical cytoplasm (Fig. 1). However, with the resolution available in the fluorescent microscope we cannot rule out that BSC merely overlays the apical surface (microvilli) and is not actually intracellular. This distinction will probably require electron microscopic studies, which are planned. (Goblet cells, which others have reported to contain SC, are negative, which we consider to be indicative of the high quality of our reagents.) Alpha chains are distributed exactly the same as BSC (and also, of course, in plasma cells). Our interpretation is that IgA secreted by lamina propria plasma cells passes into the cytoplasm of the overlying epithelial cells where it complexes with FSC. /Fully assembled secretory/ IgA is then elaborated by the epithelial cells.

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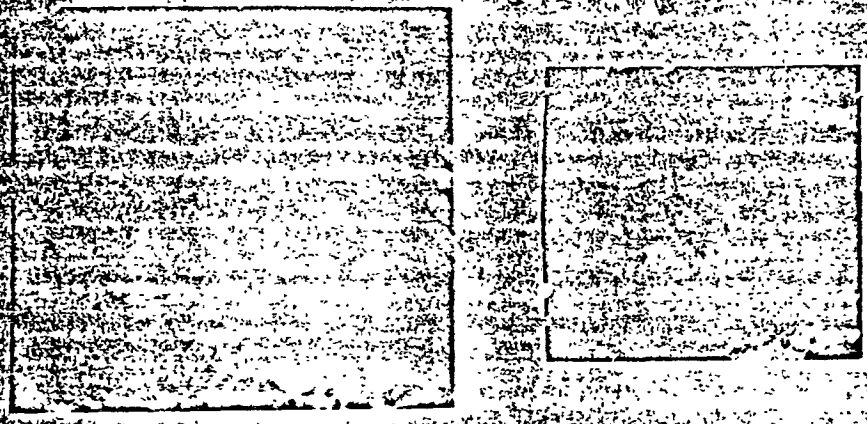

Fig. 1. Fluorescent antibody staining for SC in mucosa. Left--FSC in Golgi and apical portions of epithelial cells. Right--BSC in apices of epithelial cells.

3. Cellular studies

In terms of its cellular components, the secretory immune system has several distinctive characteristics which differentiate it from the more classical humoral antibody system. Consequently, the data now available on the surfaces, intracellular contents, and migratory patterns of B lymphocytes, which have been obtained mostly from spleen, pooled lymph node cells, bone marrow, blood, and thoracic duct lymph, may not hold for the secretory immune system. We therefore want to focus on certain aspects of B lymphocytes as they specifically relate to mucous membranes and for which there are no data in the literature.

It is well known that IgA-containing immunocytes are preponderant in mucous membranes as compared with immunocytes containing other immunoglobulin classes (1, 9-11). Studies designed to explore possible mechanisms responsible for this phenomenon will be carried out in collaboration with Dr. Julia Phillips-Quagliata of our department.

Classes of immunoglobulins are distinguished by differences in their C_H regions, the portions of immunoglobulins which are responsible for their so-called biological properties or effector functions such as complement fixation, sensitization for anaphylaxis, cytophilia, etc. The hypothesis that we want to test is that the C_H regions of immunoglobulins on B lymphocytes influence their migration and homing patterns. Specifically we want to know whether IgA on the surface of certain B lymphocytes is responsible for the predominance in mucous membranes of IgA immunocytes, perhaps because of an interaction with a particular receptor (such as SC, which is synthesized by the lining epithelial cells). Or alternatively could B lymphocytes be induced to differentiate into IgA-immunocytes because of an interaction with a local receptor such as SC? We shall use mice because of the availability of inbred strains for cell transfers, well characterized differentiation antigens, and the availability of multiple myeloma proteins.

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a. Nature of surface immunoglobulins on lymphocytes from mesenteric nodes and Peyer's patches

The first question we would like to explore is the nature of the immunoglobulins on the surfaces of lymphocytes in mesenteric nodes and Peyer's patches. We have chosen these sites for our studies of mucous membranes for convenience since bronchial lymph nodes in mice are much smaller and an analogue for Peyer's patches in pulmonary tissue is not well established. There is good evidence that the predominant cell surface immunoglobulin of mouse B lymphocytes from lymph nodes and spleen is IgM (14,15) and that bone marrow cells have surface IgG as well (16). Fixed Peyer's patch cells (rabbit) have been found by immunofluorescence not to contain intracellular immunoglobulin, including IgA (17). However, for studies of surface immunoglobulin viable cells should be used (18), and to my knowledge viable lymphocytes from mesenteric nodes and Peyer's patches have not been critically examined for surface immunoglobulins. We would like to see if perhaps IgA is better represented on such cells. We shall use cytotoxic tests and immunofluorescence and/or autoradiography after incubating cell suspensions with specific radioiodinated (^{125}I) antisera (19). These studies are underway.

b. Homing experiments

Working with rats, Gowans and Knight (20) showed that large dividing thoracic duct lymphocytes injected intravenously home to intestinal lymphoid tissue. Griscelli et al. (21), also with rats, observed a difference in the homing behavior of the large dividing lymphocytes obtained from mesenteric vs. peripheral nodes. The former tended to go to intestinal and the latter to peripheral lymphoid tissues. In studies with rabbits Craig and Cebra (22) demonstrated that lymphoid cells from Peyer's patches can seed the intestinal lymphoid tissue more effectively than cells from peripheral nodes and in addition can proliferate and differentiate into IgA-immunocytes. In the experiments with rats cited above the cells which home to the gut were not studied for their potential to form different classes of immunoglobulin, and in neither species was the nature of the immunoglobulin on the surfaces of the cells which homed to the gut examined.

We have been doing analogous homing experiments in Balb/c mice with lymphoid cells that are labelled with both ^{51}Cr (23) and ^{125}I UDR. The latter labels only large lymphocytes, which when obtained from mesenteric, as compared to peripheral, nodes preferentially home to mesenteric nodes and small intestine. This homing pattern is not so distinct with the ^{51}Cr label. We now want to use combined autoradiography and immunofluorescence (19) to examine whether cells which home to mucous membrane-associated lymphoid tissue are mainly IgA-type lymphocytes in terms of both their cell surface immunoglobulin at the time of transfer and their cell surface and intracellular immunoglobulin after tissue localization. Cell surface immunoglobulin will be studied on live cells in suspension and intracellular immunoglobulin after fixation (18). The population which homes to mucous membrane-associated lymphoid tissue will also be characterized by other means. Thus, before intravenous injection radiolabeled cells will be treated with class specific, anti-kappa, or anti-theta antisera plus complement, or F(ab')$_2$ fragments of the above without complement, or will be depleted of complement receptor lymphocytes (24). Experiments will also be done with lymphocytes from neonatally thymectomized animals. In addition to ^{125}IUDR and ^{3}H-thymidine, which label only dividing cells, we shall also use ^{3}H-uridine and ^{51}Cr which will label most of the cells to be injected. Chromium-labeled cells cannot be followed by autoradiography but offer the advantage of simplicity of counting.

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In addition to efforts to interfere with selected populations of lymphocytes in vitro prior to injection into recipients, attempts to interfere with homing by treating the recipient mice will also be made. Thus, if homing to mucosal lymphoid tissues depends on the interaction between exposed Fc regions of cell surface Ig, and a receptor, it might be possible to interfere with homing either by passively flooding the recipients with IgA (to compete with cell surface IgA for the putative receptor) or by injecting pepsin treated anti-SC antiserum into the recipient in an effort to prevent mucous membrane SC, postulated to be the receptor, from interacting with cell surface IgA. I am not aware of mouse SC having been purified, and at the moment we have no mouse SC with which to prepare antiserum. It might be easier to purify a small amount of SC from rat, rather than mouse, colostrum and use this as antigen if it cross reacts strongly. As a short-cut we have prepared chicken anti-serum against our purified human SC and shown that among mouse proteins in serum and colostrum it is also specific for SC.

Some of the experiments described above are already in progress. Since the homing phenomenon is more apparent among those lymphocytes which are synthesizing DNA (^{125}IUDR label) as compared to the general population (^{51}Cr label), we also want to do experiments on cells which have been exposed to T cell and/or B cell mitogens (25), which will increase the dividing population.

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Significance

A large body of evidence indicates that secretory immunoglobulins are of particular significance for defense against exogenous infectious agents. Furthermore, the secretory immune system has certain features which set it apart from the humoral immune system and suggest that it be considered separately. Among these novel features is the occurrence of IgA in a special form, secretory IgA, the cellular basis of which is local synthesis within the mucous membrane by two different cell types, immunocytes which make alpha, L and J chains, and epithelial cells which make the SC. The research proposed in this application seeks to enhance our understanding of some of the key features of this secretory immune system. The chemical studies are designed to explore the structure and mode of attachment of the SC component of secretory IgA, the portion of the molecule which is unique in terms of general immunoglobulin structure because it is normally absent from other immunoglobulins, including serum-type IgA, and is synthesized by epithelial cells rather than immunocytes. The studies in vivo will focus on the cellular basis for the predominance of IgA in mucous membrane secretions, namely the large numbers of IgA-type immunocytes, which are responsible for the local synthesis of IgA. We hope to gain insights into the mechanisms underlying this phenomenon and will test the hypothesis that SC serves as a local receptor which causes lymphocytes with IgA on their surface to localize in mucous membranes.

A number of different chronic respiratory diseases such as bronchitis, asthma, and emphysema are either infectious in origin or are rendered more serious by secondary infection. In these diseases damage to tissues can be brought about through the direct action of microorganisms or their products or as a result of the reactive inflammatory process on the part of the host, e.g. release of lysosomal enzymes from leukocytes. Autoimmune mechanisms may also play a role. Hopefully, a better understanding of the special features of the secretory immune system will enhance the possibilities for manipulating it so as to improve the treatment and prevention (e.g. by local immunization) of many infectious and perhaps also atopic diseases.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location).

The general facilities of New York University Medical Center are available, including adequate animal quarters. My own laboratory includes 700 square feet of space and the following major equipment: Spinco Model 120C amino acid analyzer, Zeiss PMQ II spectrophotometer, fraction collectors, chromatography cabinet, ovens, high voltage electrophoresis tanks and power supply, Spinco L-2 65 ultracentrifuge, Sorvall RC2B refrigerated centrifuge, Radiometer pH-stat and recorder, and two balances. Additional equipment and facilities are available in other laboratories of the Department of Pathology as may be needed, e.g., electron microscopes, scintillation counters, and a small animal irradiator. Also, I am a joint owner of a Spinco amino acid sequencer and a Hewlett-Packard gas chromatograph which are located in the laboratories of Dr. E.C. Franklin in the Department of Medicine at N.Y.U.

11. Additional facilities required:

None

12. Biographical sketches of investigator(s) and other professional personnel (append):

Attached

13. Publications. (five most recent and pertinent of investigator(s), append list, and provide reprints if available).

Attached

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R: REDACTED MATERIAL

CURRICULUM VITAE

Michael Emanuel Lamm
REDACTED

Date and Place of Birth:

REDACTED

Family Status:

Education:

REDACTED

Amherst College
University of Rochester School of Medicine, M.D., with honor
Western Reserve University, M.S., Chemistry
Diplomate, American Board of Pathology

Positions Held:

1959-1962	Intern and Assistant Resident in Pathology, Institute of Pathology, Western Reserve University and University Hospitals of Cleveland
1962-1964	Senior Assistant Surgeon and Surgeon (Research Associate), U.S. Public Health Service, Section on Physical Chemistry, National Institute of Mental Health, Bethesda, Maryland
1964-1968	Assistant Professor of Pathology, New York University School of Medicine
1968	Visiting Scientist, Laboratory of R. R. Porter, Dept. of Biochemistry, University of Oxford
1968-	Associate Professor of Pathology, New York University School of Medicine

Military Service:

1962-1964	Commissioned Officer, U.S.P.H.S., Reserve Corps on active duty

Societies:

1959
1959
1966
1967
1967
1967
1967

REDACTED

Honors and Fellowships Received:

1956	National Science Foundation Summer Fellow, Atomic Energy Project, University of Rochester School of Medicine
1956-1957	Student Fellow in Pathology, University of Rochester School of Medicine
1960-1962	Resident Training Fellow in Pathology, U.S. Public Health Service, Institute of Pathology, Western Reserve University and University Hospitals of Cleveland
1966-	Career Scientist of the Health Research Council of the City of New York
1972, June	Visiting Professor of Immunology, State University of Campinas, Brazil.

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CURRICULUM VITAE

Charlotte Cunningham Rundles

Date and Place of Birth: REDACTED

Education:

REDACTED

Duke University, Durham, N.C.; B.S., Zoology-Chemistry
Columbia University, New York; M.D.

Positions Held:

1972-present Post-Doctoral Fellow, Department of Pathology, New York University School of Medicine

1969-1972 Intern and Resident in Medicine, New York University-Bellevue Medical Center

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Source: https://www.industrydocuments.ucsf.edu/docs/rxpl0000

R: REDACTED MATERIAL

CURRICULUM VITAE

Michael McWilliams

Date and Place of Birth: REDACTED

Education:

REDACTED

University of California, Davis, Calif.; B.S., Veterinary Medicine
University of California, Davis, Calif.; D.V.M., Veterinary Medicine
University of California, Davis, Calif.; Ph.D., Comparative Pathology

Honors:

Phi Beta Kappa
Phi Zeta

Positions Held:

1971-present New York University School of Medicine, Post-Doctoral Fellow, Department of Pathology

1968-1970 Capt. U.S. Air Force, School of Aerospace Med., Brooks A.F.B., Texas

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Source: https://www.industrydocuments.ucsf.edu/docs/rxpl0000

R: REDACTED MATERIAL

CURRICULUM VITAE

Marshall Poger

REDACTED

Date and Place of Birth:

Education:

REDACTED

Washington University, St. Louis, Mo.; B.A., Physics, Chemistry
Brandeis University, Boston, Mass., M.A., Chemistry
Univ. of Tennessee, Memphis; M.D.

Positions Held:

1971 Resident (Pathology), New York University Medical Center
1970-71 Intern (Medicine and Pathology), New York University Medical Center

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Source: https://www.industrydocuments.ucsf.edu/docs/rxpl0000

13. Five most recent publications:

1) Pincus, C.S., Lamm, M.E. and Nussenzweig, V. Regulation of the immune response: suppressive and enhancing effects of passively administered antibody. J. Exp. Med., 133:987, 1971.

2) Eden, A., Lamm, M.E. and Nussenzweig, V. Complementarity of H and L chains from anti-hapten antibodies of different classes. J. Immunol., 108:1605, 1972.

3) Lamm, M.E. and Greenberg, J. Human secretory component: Comparison of the form occurring in exocrine immunoglobulin A with the free form. Biochemistry, 11:2744, 1972.

4) Lamm, M.E. and Frangione, B. Intrachain disulfide bridges of rabbit immunoglobulin light chains of allotypes b4 and b5. Biochem. J., 128:1357, 1972.

5) Lamm, M.E., Koo, G.C., Stackpole, C.W. and Hämmerling, U. Hapten-conjugated antibodies and visual markers used to label cell surface antigens for electron microscopy: an approach to double labeling. Proc. Nat. Acad. Sci. U.S.A., 69:3732, 1972.

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Source: https://www.industrydocuments.ucsf.edu/docs/rxpl0000

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14. First year budget.

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
Michael E. Lamm, M.D.	50%	
Charlotte Cunningham-Rundles, M.D.	100%	
Michael McWilliams, Ph.D.	100%	
Marshall Pogen, M.D.	50%	
Technical		
Stephanie Rais (research technician)	100%	
Alexandria Warner (laboratory assistant--glassware washing)	50%	
New York Univ. Med. Ctr. fringe benefits		
	Sub-Total for A	REDACTED
B. Consumable supplies (by major categories)		
colostrum at $1 per oz.		1,000
mice: 2,000 at $.80 + 2.5c per day maintenance for 1 mo.		3,100
rabbits: 40 each at $7 + 34c/day maintenance for 2 mo.		1,096
glass and plasticware		2,000
chemicals, including radiochemicals		3,500
miscellaneous supplies		500
	Sub-Total for B	11,196
C. Other expenses (itemize)		
travel (to attend scientific meetings)		300
service contracts for Spinco ultracentrifuge (L265) and amino acid analyzer (120c)		1,030
miscellaneous repairs and maintenance		300
scientific books and journals related to project		200
duplicating and laundry charges		300
	Sub-Total for C	2,130
	Running Total of A + B + C	28,597
D. Permanent equipment (itemize)		
Shandon Cyto-Centrifuge (to prepare from small amounts of sample suspensions cell monolayers for microscopic study)		890
Spinco #30 ultracentrifuge rotor (to work up large batches of colostrum)		895
	Sub-Total for D	1,785
E. Indirect costs (15% of A+B+C)	E	4,290
	Total request	34,672

15. Estimated future requirements.

	Salaries	Consumable Suppli	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	DACTED	11,500	2,230	-	4,533	34,752
Year 3		11,800	2,330	-	4,790	36,723

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Source: https://www.industrydocuments.ucsf.edu/docs/rxpl0000

16. Other sources of financial support.
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Studies on Secretory Immunoglobulin	National Institutes of Health (AI-09738)	~$55,000 per year	

N.B. The present application and the one to the NIH are similar. If both shall be funded, I shall accept only one.

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Michael E. Lamm, M.D.

Signature Michael E. Lamm Date 5/15/73

Telephone 212 (Area Code) 679-3200 (Number) X2888 (Extension)

Checks payable to

New York University Medical Center
Attention: Mr. John Ballow

Mailing address for checks

550 First Avenue

New York, New York 10016

Responsible officer of institution

Typed Name Ivan L. Bennett, Jr., M.D.

Title Director

Signature [signature] Date 5/15/73

Telephone 212 (Area Code) 679-3200 (Number) X3977 (Extension)

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